Iran. J. Ichthyol. (March 2014), 1(1): 7–11
Received: February 10, 2014
Accepted: March 20, 2014

Application of Ovaprim in artificial reproduction of Eurasian perch, *Perca fluviatilis* L. under controlled conditions

Dariusz KUCHARCZYK[1*], Katarzyna TARGOŃSKA[1], Rafał CHWALUCZYK[2]

[1]*Department of Lake and River Fisheries, University of Warmia and Mazury in Olsztyn, ul. Oczapowskiego 5, 10-718 Olsztyn-Kortowo, Poland*
[2]*Czarci Jar Fish Farm, Poland*

Email: dariusz.kucharczyk@uwm.edu.pl

Abstract: Eurasian perch, *Perca fluviatilis* L., is one of the most important species in European freshwater aquaculture and probably one of most important of cold freshwater species in nearest future. The aim of this study was to test the Ovaprim in artificial reproduction in Eurasian perch in two temperatures (12 and 14°C) with using new oocyte maturation stage system. The results obtained in present study showed that the oocyte maturity stage is very important in artificial reproduction of perch. The spawning effectiveness, in present study described as ovulation rate, latency time and embryo survival to the eyed-egg-stage, was higher when fish with higher oocyte maturity stage was used for reproduction. The latency time was depended on oocyte maturity stage and on water temperature. If the water temperature was highest from tested (14°C) and oocyte maturity stage was also the highest (4^{th}) the latency time was the shortest. The obtained results showed that Ovaprim might be successfully applied in artificial reproduction of Eurasian perch under controlled conditions.

Keywords: Eurasian perch, Ovulation, Spawning effectiveness, Ovaprim.

Introduction

Eurasian perch, *Perca fluviatilis* L., is one of the most important species in European freshwater aquaculture and probably one of most important of cold freshwater species in nearest future (Kouril et al. 1997; Mandiki et al. 2004; Szczerbowski et al. 2009; Żarski et al. 2012). The high market price and market demand involves perch culture and building new fish farm for perch culture in Europe. The increasing interest in perch biology and management creates a need of development new and improving existing methods of obtaining a stocking material and market size fish under controlled conditions. The first step in intensive fish culture is controlled reproduction under controlled conditions. The obtaining of good quality mature gametes is important for production in aquaculture but also for special purposes, such as genetic engineering or cryopreservation (Krejszeff et al. 2009; Targońska et al. 2010). The reproduction of perch under controlled conditions is possible without the use of hormonal agents (Kucharczyk et al. 1996b, 1998), however, are most often the hormonal agents were administered due to spawning synchronization (Kouril et al. 1997; Kucharczyk et al. 2001; Szczerbowski et al. 2009; Targońska et al. 2014). Without the use of hormonal stimulation the reproductive period is very stretched in time, and the percentage of ovulating females as well as the quality of gametes at the end of the reproductive period are much lower than in the case of that the use of hormonal agents (Kucharczyk et al. 1996b, 1998, 2001). The necessities of use hormonal agents were strictly demonstrated in out-of-season spawning of Eurasian perch (e.g. Szczerbowski et al. 2009; Targońska et al. 2014).

From time to time new hormonal agents are present on the market. On the other hand, new

advanced techniques from time to time are developed and described (Krejszeff et al. 2010). In the case of Eurasian perch during last few years the system of oocyte maturity stage and quality of oocytes (before and after ovulation) was established and described (Żarski et al. 2011, 2012). The new in Eurasian perch gametes maturation process commercial preparation Ovaprim was successfully used in the out-of-season spawning (Targońska et al. 2014). But is the lack of data of use this preparation in season spawning of Eurasian perch. The aim of this study was to test the Ovaprim in artificial reproduction in Eurasian perch in two temperatures (12 and 14°C) with using new oocyte maturation stage system.

Materials and methods

Eurasian perch breeders were collected from earthen pond in Czarci Jar Fish farm (North Poland) in the late autumn 2010. Selected fish, in size 150-250 g each, were kept during winter time at this farm in indoor located tanks on natural conditions (photoperiod and water temperature) (Kujawa et al. 1999). As a food, small prey fish were offered to the spawners. During spring 2011, when the water temperature was increasing to the 8°C, the fish were marked and oocyte maturity stage was recognized from all females. For this the oocytes were taken from few females using the catheter. Oocytes were sampled in vivo and placed in Serra's solution for clarification of the cytoplasm. After 5 minutes, the position of oocytes germinal vesicle and oil droplets coalescence was determined using a 6-stages scale (Żarski et al. 2011) as following:

• Stage I – the GV is situated in the oocyte center, oil droplets were poorly visible.
• Stage II – beginning of GV migration (GV in majority was located very close to the center of oocyte), beginning of coalescence of oil droplets, which were very well visible.
• Stage III – migrating GV (reached half the oocyte diameter), oil droplets were clearly visible.
• Stage IV – the GV located above half the oocyte, a large oil droplet was well visible (the droplet diameter was greater than the GV diameter and it reaches the size of about 1/3 of the oocyte diameter) with visible smaller droplets.
• Stage V – the GV located above half the oocyte, clearly visible one large (size of about half the oocyte diameter) oil droplet.
• Stage VI – oocyte samples taken for analysis were macroscopically transparent; no visible GV after they were placed in Serra's solution (following GVBD), oocytes at the pre-ovulation stage.

For further investigation, only the females with oocytes at stages 2 and 4 were used. Then, the water temperature was raised to 10°C and the fish were received hormonal agent: Ovaprim (Syndel, Canada). Ovaprim contains 20 μg of salmon LHRH analogue (sLHRHa) [D-Arg^{6}Pro^{9}Net-sLHRH] and 10 mg of the dopamine antagonist domperidone in 1 cm^{3} propylene glycol (Peter et al. 1993). Fish from control groups received injection from 0.9% NaCl sterile solution. Intraperitoneal injections at dose 0.5 cm^{3}kg^{-1}were made at the base of the left ventral fin. After injection, fish from control groups were kept at 10°C, for other groups the water temperature was raised to 12°C (group A) or 14°C (group B). The control was carried out by two ovulation day after the last injection for the next five days. All manipulations were performed under anesthesia fish in a solution of MS-222 (150 mg L^{-1}). Eggs strands were collected to dry baskets. Later few fragments from each egg strand were sampled. Samples were subsequently inseminated in petri dishes using 0.20 ml of semen (from at least three males), and incubated in separate containers at 12-14°C, according to the method described by Kucharczyk et al. (1996a) Then, in order to determine the percentage of embryo survival, the samples were photographed10 days post fertilization (the eyed-egg stage).

Arcsine transformations were made on data expressed in percentage before subjected to statistical analysis. Then, analysis of variance

Table 1. The results of artificial spawning of Eurasian perch females which had the oocytes in 2nd maturity stage.

	Control group (10 °C)	Group A (12 °C)	Group B (14 °C)
Ovaprim dose (cm^3kg^{-1})	-	0.5	0.5
Number of females	10	10	10
Ovulation rate (%)	0	40	60
Latency time (days)	-	4.5 ±0.7 a	2.6 ±0.6 b
Embryo-survival	-	79.8 ± 2.3	74.8 ± 5.4
Female's mortality (%)	10	10	10

Table 2. The results of artificial spawning of Eurasian perch females which had the oocytes in 4th maturity stage

	Control group (10 °C)	Group A (12 °C)	Group B (14 °C)
Ovaprim dose (cm^3kg^{-1})	-	0.5	0.5
Number of females	10	10	10
Ovulation rate (%)	20	80	90
Latency time (days)	6.8 ± 0.8 a	3.2 ± 0.7 b	1.9 ± 0.3 c
Embryo-survival*	77.5 ± 3.2 b	87.4 ± 2.2 a	86.2 ± 2.1 a
Females mortality (%)	10	10	10

ANOVA was applied. If the analysis of variance showed statistically significant differences, post hoc Tuckey's test was performed at significance level α=0.05.

Results and discussion

The artificial reproduction in captivity gives the possibility to mass production of high quality larvae and juveniles. For this reason, the protocol of artificial reproduction should be done separately for each species and sometimes also for different stock or population (Kucharczyk et al. 2001; Krejszeff et al. 2009; 2010 a, b). Also, some environmental factors should be tested, like the temperature (Targońska et al. 2010).

The results obtained in present study showed that the oocyte maturity stage is very important in artificial reproduction of perch. The spawning effectiveness, in present study described as ovulation rate, latency time and embryo survival to the eyed-egg-stage, was higher when fish with higher oocyte maturity stage was used for reproduction. In females with 4th oocyte maturity stage (using scale described by Żarski et al. 2011) ovulated at level of 80-90% and produced eggs with the highest quality (embryo survival over 86%). In fish with 2nd oocyte stage, the ovulation rate was between 40 and 60% and the embryo survival was lower. It suggests, that for artificial reproduction, the females with the highest oocytes maturity stages should be selected (Tables 1, 2).

In present study low ovulation rates of females from control groups were noted (from 0 to 20%). These results are in opposite with data described e.g. by Kucharczyk et al. (1996b, 1998, 2001) for the same species. But in these research, the maturity stage of oocytes was much higher and was between 4th and 6th stage using scale described by Żarski et al. (2011) than in present study. So, it means that spontaneous spawning in captivity is possible in the case of Eurasian perch, if the maturity stage was 4th or higher. The low (10°C) temperature in control groups caused by longer latency time. On the other hand application of spawning agents on fish with 2nd oocyte maturity stage involved the ovulation in about 50% of females. The quality of gametes was high

(about 75% or over) but lower than that from females which were at 4th oocyte maturity stage. In these last, the survival rate of embryos is similar to earlier in-season studies (Kucharczyk et al. 1996b, 1998, 2001; Kouril et al. 1997).

The latency time was depended on oocyte maturity stage and on water temperature. If the water temperature was highest from tested (14°C) and oocyte maturity stage was also the highest (4th) the latency time was the shortest. The latency time 4.5 days for females which had oocytes at 2nd stage and was spawned at 12°C was similar to data obtained in out of season spawning of perch (Szczerbowski et al. 2009; Targońska et al. 2014).The survival of females were high (90%) and was higher than in other research (e.g. Szczerbowski et al. 2009; Targońska et al. 2014).

The obtained results showed that Ovaprim might be successfully applied in artificial reproduction of Eurasian perch under controlled conditions.

Acknowledgements

This study was partially supported by the projects: "Innovations in finfish aquaculture with special reference to reproduction" (InnovaFish), Operational Programme Sustainable Development of the Fisheries Sector and Coastal Fishing Areas 2007–2013 (OR14-61724-OR1400003/09/10/11).

References

Kouril, J.; Linhart, O. & Relot, P. 1997. Induced spawning of perch by means of a GnRH analogue. Aquaculture International 5: 375–377.

Krejszeff, S.; Targońska, K.; Żarski, D. & Kucharczyk, D. 2009. Domestication affects spawning of the ide (*Leuciscus idus*) - preliminary study. Aquaculture 295: 145–147.

Krejszeff, S.; Targońska, K.; Żarski, D. & Kucharczyk, D. 2010a. Artificial reproduction of two different spawn-forms of the chub. Reproductive Biology 10: 67–74.

Krejszeff, S.; Żarski, D.; Kucharczyk, D.; Kupren, K.; Targońska, K. & Mamcarz, A. 2010b. An experimental device for eggs incubation and fish larvae rearing under laboratory conditions. Polish Journal of Natural Science 25: 190–199.

Kucharczyk, D.; Kujawa, R. & Mamcarz, A. 1996a. New experimental incubation unit for eggs of the perch *Perca fluviatilis*. Progressive Fish-Culturist 58: 281–283.

Kucharczyk, D.; Kujawa, R.; Mamcarz, A.; Skrzypczak, A. & Wyszomirska, E. 1996b. Induced spawning in perch, *Perca fluviatilis* L. using carp pituitary extract and HCG. Aquaculture Research 27: 847–852.

Kucharczyk, D.; Kujawa, R.; Mamcarz, A.; Skrzypczak, A. & Wyszomirska, E. 1998. Induced spawning in perch, *Perca fluviatilis* L., using FSH + LH with pimozide or metoclopramide. Aquaculture Research 29: 131–136.

Kucharczyk, D.; Szczerbowski, A.; Łuczyński, M.J.; Kujawa, R.; Mamcarz, A.; Wyszomirska, E.; Szabo, T. & Ratajski, S. 2001. Artificial spawning of Eurasian perch, *Perca fluviatilis* L. using Ovopel. Archives of Polish Fisheries 9: 39–49.

Kujawa, R.; Kucharczyk, D. & Mamcarz, A. 1999. A model system for keeping spawners of wild and domestic fish before artificial spawning. Aquacultural Engineering 20: 85–89.

Mandiki, S.N.M.; Blanchard, G.; Melard, C.; Koskela, J.; Kucharczyk, D.; Fontaine, P. & Kestemont, P. 2004. Effects of geographic origin on growth and food intake in Eurasian perch (*Perca fluviatilis* L.) juveniles under intensive culture conditions. Aquaculture 229: 117–128.

Peter, R.E.; Lin, H.R.; van der Kraak, G. & Little, M. 1993. Releasing hormones, dopamine antagonists and induced spawning. In: Muir, J.P. & Roberts, R.J. (eds.), Recent Advances in Aquaculture, vol IV. Institute of Aquaculture, Blackwell Scientific Publications, Oxford, pp. 25–30.

Szczerbowski, A.; Kucharczyk, D.; Mamcarz, A.; Łuczyński, M.J.; Targońska, K. & Kujawa, R. 2009. Artificial off-season spawning of Eurasian perch *Perca fluviatilis* L.. Archives of Polish Fisheries 17: 95–98.

Targońska, K.; Kucharczyk, D.; Kujawa, R.; Mamcarz A. & Żarski, D. 2010. Controlled reproduction of asp, *Aspius aspius* (L.) using luteinizing hormone

releasing hormone (LHRH) analogues with dopamine inhibitors. Aquaculture 306: 407-410.

Targońska, K.; Szczerbowski, A.; Żarski D.; Łuczyński, M.; Szkudlarek, M.; Gomułka, P. & Kucharczyk, D. 2014. Comparison of different spawning agents in artificial out-of-season spawning of Eurasian perch, *Perca fluviatilis* L. Aquaculture Research (published online doi:10.1111/are.12010)

Żarski, D.; Bokor, Z.; Kotrik, L.; Urbanyi, B.; Horváth, A.; Targońska, K.; Krejszeff, S.; Palińska, K. & Kucharczyk, D. 2011. A new classification of a preovulatory oocyte maturation stage suitable for the synchronization of ovulation in controlled reproduction of Eurasian perch, *Perca fluviatilis* L. Reproductive Biology 11(3): 194-209.

Żarski, D.; Krejszeff, S.; Horváth, Á.; Bokor, Z.; Palińska, K.; Szentes, K.; Łuczyńska, J.; Targońska, K.; Kupren, K.; Urbányi, B. & Kucharczyk, D. 2012. Dynamics of composition and morphology in oocytes of Eurasian perch, *Perca fluviatilis* L., during induced spawning. Aquaculture 364-365: 103-110.